

## Copper, Rhodanine Stain Kit - Technical Memo

### KIT INCLUDES:

Solution A: Rhodanine Stock Stain 0.2%, Alcoholic	Part 9113A 50 ml
Solution B: Hematoxylin Stain, Mayer Modified	250 ml
Solution C: Sodium Borate 0.5%, Aqueous	500 ml

**COMPLIMENTARY POSITIVE CONTROL SLIDES:** Enclosed with this kit are two complimentary unstained positive control slides to be used for the initial verification of staining techniques and reagents. Verification must be documented by running one Newcomer Supply complimentary positive control slide along with your current positive control slide for the first run. Retain the second complimentary control slide for further troubleshooting, if needed.

*Individual stain solutions and additional control slides may be available for purchase under separate part numbers at [www.newcomersupply.com](http://www.newcomersupply.com).*

### Additionally Needed:

Xylene, ACS	Part 1445
Alcohol, Ethyl Denatured, 100%	Part 10841
Alcohol, Ethyl Denatured, 95%	Part 10842
Coplin Jar, Plastic	Part 5184 (for microwave modification)

**For storage requirements and expiration date refer to individual bottle labels.**

### APPLICATION:

Newcomer Supply Copper, Rhodanine Stain Kit procedure, with included microwave modification, is used for the detection of copper in tissue sections. Abnormal copper accumulations are predominantly found in liver tissue, most notably in Wilson's disease.

### METHOD:

**Fixation:** Formalin 10%, Phosphate Buffered (Part 1090)

**Technique:** Paraffin sections cut at 5 microns

**Solutions:** All solutions are manufactured by Newcomer Supply, Inc.

All Newcomer Supply Stain Kits are designed to be used with Coplin jars filled to 40 ml following the staining procedure provided below. Some solutions in the kit may contain extra volumes.

### STAINING PROCEDURE:

1. Deparaffinize sections thoroughly in three changes of xylene, 3 minutes each. Hydrate through two changes each of 100% and 95% ethyl alcohols, 10 dips each. Wash well with distilled water.
  - a. See Procedure Notes #1 and #2.
2. Prepare Working Rhodanine Solution and mix well.
  - a. Shake Solution A: Rhodanine Stock Stain 0.2%, Alcoholic well before use.
  - b. Solution A: Rhodanine Stock Stain 0.2%, Alcoholic 3 ml
  - c. Distilled Water 47 ml
3. Stain slides in Working Rhodanine Solution at 37°C for 18 hours.
 

**Microwave Modification:** See Procedure Note #3.

  - a. Place slides in a plastic Coplin jar containing Working Rhodanine Solution and microwave for 6 minutes at 70°C.

At the end of incubation (for both 37°C and microwave), to avoid unwanted slide precipitate, pour off warm Working Rhodanine Solution into a second Coplin jar; reserve and set aside.
4. Rinse slides well in several changes of distilled water. Check microscopically to determine adequate copper/reddish brown development in positive control slide. Return slides to reserved Working Rhodanine Solution if additional incubation is required.
5. Prepare dilute Mayer Hematoxylin Stain Solution and mix well:
  - a. Solution B: Hematoxylin Stain, Mayer Modified 20 ml
  - b. Distilled Water 20 ml
6. Stain in dilute Mayer Hematoxylin Stain Solution for 10 minutes.
  - a. See Procedure Note #4.
7. Rinse in distilled water.

8. Rinse slides in Solution C: Sodium Borate 0.5%, Aqueous; 2-3 quick dips.
9. Rinse well in distilled water.
10. Dehydrate in two changes each of 95% and 100% ethyl alcohol. Clear in three changes of xylene, 10 dips each; coverslip with compatible mounting medium.

### RESULTS:

Copper	Copper/reddish brown
Nuclei	Light blue

### PROCEDURE NOTES:

1. Drain staining rack/slides after each step to prevent solution carry over.
2. Do not allow sections to dry out at any point during staining procedure.
3. The suggested microwave procedure has been tested at Newcomer Supply using an "EB Sciences", 850 watt microwave oven with temperature probe and agitation tubes. This procedure is reproducible in our laboratory. It is nonetheless a guideline and techniques should be developed for your laboratory which meet the requirements of your situation. Microwave devices should be placed in a fume hood or vented into a fume hood, according to manufacturer's instructions, to prevent exposure to chemical vapors.
4. Copper may be masked if over-stained with hematoxylin.
5. If using a xylene substitute, closely follow the manufacturer's recommendations for deparaffinization and clearing steps.

### REFERENCES:

1. Carson, Freida L., and Christa Hladik. *Histotechnology: A Self-Instructional Text*. 3rd ed. Chicago, Ill.: American Society of Clinical Pathologists, 2009. 271-273.
2. Sheehan, Dezna C., and Barbara B. Hrapchak. *Theory and Practice of Histotechnology*. 2nd ed. St. Louis: Mosby, 1980. 230.
3. Modifications developed by Newcomer Supply Laboratory.